Current literature highlights – July 2001

HIV Protease Inhibitors

The human immunodeficiency virus type 1 (HIV-1) is the etiologic agent of acquired immunodeficiency syndrome (AIDS). The discovery of clinically effective HIV protease inhibitors has significantly improved the lifestyles of many individuals afflicted with the virus. Nevertheless, current protease inhibitors suffer to some extent from issues not limited to first-pass metabolism, toxicities and food restrictions which often contribute to patient non-compliance. Recently the emergence of multi-drug resistant viral variants has been confirmed, further compromising the effectiveness of current PI therapy. In an effort to produce compounds possessing improved pharmacokinetic and potency profiles, a combinatorial library of compounds targeting HIV protease inhibition was synthesised (Combinatorial diversification of indinavir: in vivo mixture dosing of an HIV protease inhibitor library, T. A. Rano *et. al., Bioorg. Med. Chem. Lett.*, 10, (2000), 1527-1530). A library of 60 compounds was synthesised in mixtures of 20 on Rapp TentaGel S CO_2H resin.

These compounds were tested in mixtures of 20 using in vivo dosing of multiple component mixtures, for their ability to prevent cleavage of a substrate by the protease enzyme (IC_{50}) and to inhibit the spread of viral infection in MT4 human T-lymphoid cells infected with the IIIb isolate (CIC_{95}). Of those mixtures tested, the most active gave an enzyme inhibition IC_{50} of 1.1 nM, and a value for the inhibition of viral spread (IIIb) CIC_{95} of 200 nM. The success of this library is in providing a step forward towards the identification of a second generation HIV protease inhibitor possessing improved metabolic and potency profiles.

Cell-permeable molecules

Small molecules that induce or stabilise the association of macromolecules hold promise as biological effectors. Such 'chemical inducers of dimerisation' (CIDs) are well known in Nature; anti-cancer agents adriamycin and etoposide act by stabilising a catalytic intermediate having topoisomerase II bound to its nicked DNA substrate. The immunosuppressive agent FK506 induces the dimerisation of FK506-binding protein (FKBP) and calcineurin (Cn), thereby abrogating the latter's protein phosphatase activity. The potential of designed synthetic CIDs to bring together signalling proteins in a spatially and temporally controlled manner gives the ability to engineer a variety of inducible signal transduction processes into cells. These synthetic 'dimerisers' all involve bifunctional molecules having two ligand moieties linker together, each of which binds a known receptor. There is also the possibility to induce the association of one known macromolecule with an unknown one, thereby creating biological relationships that nature may not have explored. Dimerising two known macromolecules can present a challenge when a ligand is known for one partner but not the other. Both of these lines of research could be pursued by the construction of bifunctional small-molecule libraries having an invariant ligand attached to a diversified ligand whose structure varies among the members of the library. A combinatorial chemistry approach was used to construct the first such diversified library of synthetic candidate heterodimerisers (A synthetic library of cell-permeable molecules, G. L. Verdine *et. al.*, *J. Am. Chem. Soc.*, 123, (2001), 398-408). A library of 320 individual compounds was synthesised on Trityl-chloride solid phase resin. For synthetic heterodimerisers to be useful in biological assays with intracellular targets, they must

be cell-permeable. To assess the cell permeability of these library compounds, cells that express two FKBP fusion proteins - one containing the ZFHD1 DNA-binding domain and the other containing the p65 acidic transcriptional activator domain - were used. The small-molecule heterodimer AP1889 brings these two protein components together, thereby activating transcription of the downstream encoding secreted alkaline phosphatase (SEAP). A randomly chosen sub-set of the library comprising 25 heterodimers was screened separately at 500 nM concentration. Each of the compounds tested strongly inhibited SEAP expression, indicating cell-permeability and suitability for use in biological screens. Future research is directed at determining whether any of these 320 library members will function as heterodimers, allowing the possibility of engineering a variety of inducible signal transduction processes into cells. Studies are ongoing in a variety of biological assay systems to address these aims.